REMARKS

Power of Attorney

Please note that a Power of Attorney with Revocation of Previous Power is submitted concurrently with this Amendment.

Restriction Requirement

Applicants continue to traverse the election requirement. Applicants reaffirm the election of the claims of Group I, but it is urged that the claims of Groups II, III, IV and VI should be joined with the claims of Group I. Applicants do not traverse the splitting of the claims of Group V from the claims of Group I.

The Office Action states that inventions "are unrelated if it can be shown that they are not disclosed as capable of use together and they have different modes of operation, different functions, or different effects (MPEP § 806.04, MPEP § 808.01)." Although the Office Action has stated, e.g., that the inventions of I and II-IV are different because they have different modes of operation, different functions and different effects, that statement merely addresses the second portion of the requirement for imposing a restriction requirement and Applicants do not agree with the statement. Furthermore, it has not addressed the first requirement which is that the stated different inventions "are not disclosed as capable of use together". It is urged that if inventions are disclosed as being capable of being used together then a restriction requirement is improper. It is asserted that the invention of Groups I-IV and VI are all capable of being used together.

The claims of Group I are drawn to a diagnostic method for determining whether an individual has a disease associated with the presence of antiphospholipid antibodies by detecting the presence of absence of lipidic particles in a serum sample from the individual. The claims of Group II are drawn to a kit for detecting anti-lipidic particle antibodies which are present in an individual suffering from a disease associated with antiphospholipid antibodies. This kit is used to determine whether antibodies to lipidic particles are present and thus indirectly whether lipidic particles are present which is the purpose of the method of the claims of Group I. The claims of Group III are drawn to a kit for detecting anti-lipidic particle antibodies in a sample from an individual suffering from a disease associated with antiphospholipid antibodies and is a kit for the same purpose as the kit of Group II. The claims of Group IV are drawn to a kit for

detection of lipidic particles in a sample from an individual suffering from a disease associated with antiphospholipid antibodies and is a kit to be used for practicing the method of the claims of Group I. The claims of Group VI are drawn a kit for determining different cellular physiologic states in a sample of cells. The kit of the claims of Group VI comprises antibodies to anti-lipidic particles as do the claims of Groups II and IV. The heart of the "invention" of group VI lies not in the buffer or fluorescent or enzymatic means but in the anti-lipidic particle monoclonal antibody, although the claimed invention is the combination with the buffer and fluorescent or enzymatic means.

The inventions of Groups I and II-IV and VI are related because they are disclosed as being capable of being used together and they have similar modes of operation, as all of them utilize a first antibody (anti-lipidic particle antibody) to be bound to lipidic particles, a second antibody conjugated to enzymes or fluorochromes and to be bound to the first antibody, as a detectable-labeled reagent useful for detecting the binding of the first antibody to the lipidic particles. They have similar functions, as all of them are directed to the detection of lipidic particles from a sample from an individual, and they have similar effects, as all of them allow the diagnosis of whether an individual under consideration is developing an illness associated with the presence of antiphospholipid antibodies, even though such an individual does not yet present anti-cardiolipin antibodies, lupus anti-coagulant, anti-DNA or anti-nuclear antibodies.

To summarize, the claims of Group I are method claims for determining whether an individual has a disease associated with the presence of antiphospholipid antibodies and the claims of Groups II-IV and VI are all kits to be used for exactly that purpose. It is therefore urged that the claims of Groups I-IV and VI are capable of being used together and consequently should not be subjected to a restriction requirement. It is requested that the restriction requirement splitting the claims of Group I from the claims of Groups II-IV and VI be withdrawn.

Specification

The Office Action stated that the application was replete with terms which are not clear, concise and exact and some examples were presented in the Office Action. Those examples specifically pointed to have been amended if found and numerous other changes have been made to the specification. Concerning the statement in the Office Action that the phrase "make react with the cells" is found on page 10, line 14, the undersigned does not see that phrase at that

location. The undersigned would appreciate if the Examiner would check this because if the phrase is found by the Examiner at that location it means that the Examiner's copy of the application and the undersigned's copy of the application are different.

The Office Action states that there are contradictions within the specification such as page 12, lines 13-23. The alleged contradiction is that one paragraph discloses the detection of the presence or absence of lipidic particles in a serum sample while the next paragraph discloses detection of antibodies in a serum sample by using antigen lipidic particles. Applicants fail to see any contradiction. One aspect of the invention is a method to determine whether a serum sample contains lipidic particles and to associate this with an antiphospholipid syndrome. A second aspect of the invention is to determine whether antibodies to lipidic particles are present in a sample, again to associate this presence with an antiphospholipid syndrome.

Drawings

Applicants take note of the fact that formal drawings will be required upon allowance of the application.

Information Disclosure Statement

Applicants appreciate the statement concerning the statement regarding a proper Information Disclosure Statement. Such a statement is transmitted concurrently with this Amendment.

Claims

All of the claims have been canceled and replaced with new claims 32-90. Original claims 22-25 are canceled without prejudice to refiling such claims as part of a divisional application. The remainder of the claims have been canceled but rewritten as shown in the new claims. It was felt that by canceling the original claims and representing them in a logical order as amended with the several newly added dependent claims that the claims would be simpler to follow. The correspondence between the originally filed claims and the newly presented claims is as follows:

Original claims 1-5 correspond to new claims 32-45.

Original claims 6-15 correspond to new claims 46-59.

Original claims 16-21 correspond to new claims 60-71.

Original claims 26-31 correspond to new claims 72-90.

Support for the new claims is to a large extent found in the claims as originally filed. Further support for the claims is as follows:

Claim 32 - page 11, lines 12-34 and page 12 lines 13-23.

Claims 33, 40, 47, 61, 73 and 80 - page 20, lines 1-4.

Claims 35, 42, 54, 67, 75 and 86 - page 30, lines 6-10 and Table 2 on page 32.

Claims 36, 43, 55, 68, 76 and 87 - page 17, lines 19-23; page 19, lines 2-9; page 30, lines 6-10; and Table 2 on page 32.

Claims 37, 44, 56, 69, 77 and 88 - page 18, lines 12-13; page 24, lines 3-6, page 30, lines 6-10; and Table 2 on page 32.

Claim 39 - page 11, lines 12-34; page 12, lines 13-23.

Claim 46 - page 23, lines 6-34; page 24, lines 1-8.

Claim 60 - page 23, lines 6-34; page 24, lines 1-8.

Claim 72 - page 44, lines 11-23; page 45, lines 31-33; page 46, lines 1-6.

Claim 79 - page 48, lines 23-34.

Rejections Under 35 U.S.C. § 112, first paragraph

Claims 1-5 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabling for all lipidic particles. The Office Action noted that lipidic particles are always present in the serum. The claims have been amended so as not to refer to serum. Instead they refer to samples such as erythrocytes, leukocytes, etc. Additionally, the lipidic particles relating to the claims differ from the miscellaneous lipidic particles circulating throughout the bloodstream. The lipidic particles of interest are defined on page 5, lines 1-5, as "Lipidic arrangements in hexagonal II or micellar phases ..." This definition distinguishes the lipidic particles of the claims from other lipidic particles.

Claims 1-5 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabled. The claims have been amended so as not to refer to serum, as discussed above, and now refer to, e.g., liposomes, erythrocytes, leukocytes, plaquettes and neoplastic cells.

In view of the amendments to the claims, it is urged that the claims as now pending are enabled and it is requested that the rejections under 35 U.S.C. § 112, first paragraph, be withdrawn.

Rejections Under 35 U.S.C. § 112, second paragraph

Claims 1-5 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. The claims have been amended to address these issues and it is urged that the amended claims are definite. It is requested that the rejections under 35 U.S.C. § 112, second paragraph, be withdrawn.

Rejections Under 35 U.S.C. § 102(b)

Claims 1-3 were rejected under 35 U.S.C. § 102(b) as being anticipated by Maxfield Wilson et al. In the Maxfield Wilson method, the "liposome reagent comprises a liposome, a ligand chosen to bind specifically to the analyte and associated with the liposome membrane, and a haptenated component associated with the membrane of the liposome, where the hapten is chosen to bind specifically to a receptor on a solid phase of the assay, or to a component of the signal detection system used in the assay" (Col. 2, lines 47-54). The analytes detected can be: "proteins, including antibodies, antigens, nucleic acids, steroids, hormones and the like" (Col. 6, lines 12-13). The anti-phospholipid antibodies refers "to antibodies which generally bind to negatively charged phospholipids, including cardiolipin (diphosphatidylglycerol), phosphatidylserine, phosphatidylinositol and phosphatidic acid" (Col. 6, lines 2-6).

Besides, in Maxfield Wilson et al. USP 5780319 the ligand chosen is "a negatively charged phospholipid, including cardiolipin, phosphatidylserine, phosphatidylinositol and phosphatidic acid" (Col. 6, lines 2-8).

On page 5, lines 1-5 of the specification of the present application a clear definition is given as follows: "Lipidic arrangements in hexagonal II or micellar phases, as well as any other structural arrangement of lipids that do not form a bilayer but that is immerse in a bilayer, are considered, for the purposes of this invention, as lipidic structures different to the lipidic bilayer or 'lipidic particles', independently of the kind of lipids that are forming these structures".

In addition, "lipidic particles are formed incubating liposomes with an effective amount of the lipidic particles inducer agent (divalent cations: Ca or Mn, or drugs producing lupus

induced by drugs in humans: chlorpromazine or procainamide) at a temperature between 25 to 40°C" (page 14, lines 4-5 of the specification). Thus, liposomes used in the present invention are liposomes bearing lipids in a molecular arrangement different from the bilayer arrangement, or "lipidic particles", immersed in their bilayer membranes. These liposomes are different from those used in the liposome reagent of Maxfield Wilson's Patents 5,776,487 and 5,780,319 where molecular arrangements different from the bilayer are not considered.

Furthermore, in the Maxfield Wilson method, the liposome reagent does not have lipidic particles in their liposomes. Therefore this liposome reagent can not detect the anti-lipidic particle antibodies described in the present invention. In the present method, liposomes or neoplastic cells bearing lipidic particles react with the anti-lipidic particles antibodies from a patient's sample, therefore, these antibodies are different from the antibodies detected in Patent 5,776,487 with Maxfield Wilson's liposome reagent. In Patent 5,776,487 the anti-phospholipid antibodies refer to antibodies which generally bind to negatively charged phospholipids, including cardiolipin, phosphatidylserine, phosphatidylinositol and phosphatidic acid; however, the molecular arrangement of these lipids in its liposome reagent is not taken into consideration.

Claims 1, 2, 4 and 5 were rejected under 35 U.S.C. § 102(b) as being anticipated by Stewart et al. In U.S. Patent 5,840,587 phospholipid coated particles "include particles of suitable shape composed of a material capable of holding a charge such as polypropylene, polyethylene, acrylonitrile, polycarbonate or nitrocellulose or magnetic beads" (Col. 4, lines 9-11). "Polystyrene microspheres are obtainable in a variety of sizes and are preferred for preparation of the phospholipid coated particles of the invention" (Col. 4, lines 4-6). In addition, "The phospholipid coated microspheres of the invention provide a convenient antigen-presenting device which may be employed along with a variety of detecting procedures to detect and to determine antiphospholipid antibodies" (Col. 4, lines 49-52).

However, on page 5, lines 1-5 of the specification of the present application a clear definition is given as follows: "Lipidic arrangements in hexagonal II or micellar phases, as well as any other structural arrangement of lipids that do not form a bilayer but that is immerse in a bilayer, are considered, for the purposes of this invention, as lipidic structures different to the lipidic bilayer or "lipidic particles", independently of the kind of lipids that are forming these structures".

In addition, "lipidic particles are formed incubating liposomes with an effective amount of the lipidic particles inducer agent (divalent cations: Ca or Mn, or drugs producing lupus induced by drugs in humans: chlorpromazine or procainamide) at a temperature between 25 to 40°C" (Page 14, Lines 4-5 of the specification). Therefore, liposomes used in the present invention are liposomes bearing lipids in a molecular arrangement different from the bilayer arrangement, or "lipidic particles", immersed in their bilayer membranes. These liposomes are clearly different from the phospholipid coated microspheres used in USP 5,840,587.

Furthermore, assays employing liposomes, which are experimental models of cell membranes, have the advantage of presenting lipids in a more native molecular arrangements: bilayer and lipidic particles, than phospholipid coated microspheres, where lipids are bound directly to the solid phase of the microspheres probably in a molecular arrangement different from that found in cell membranes. In consequence, the antiphospholipid antibodies detected with both antigens: phospholipid coated microspheres used in USP 5,840,587, and liposomes bearing lipidic particles used in the present invention (page 13 lines 23-25), probably have a different specificity because the method of the present invention identifies antiphospholipid antibodies in a different way when compared to that of USP 5,840,587. Therefore, these different antiphospholipid antibodies may generate different monoclonal antibodies. The monoclonal antibody H308-APmAb, described in the present invention (page 15 lines 3-34, page 16 lines 1-6, page 58 line 5), showed immunoreactivity only with lipids associated with lipidic particles in liposomes and do not have any reactivity with lipids associated with bilayers (page 40 lines 17-20).

In view of the above arguments, it is urged that the prior art does not anticipate the pending claims and it is requested that the rejections under 35 U.S.C. § 102(b) be withdrawn.

In view of the above arguments, it is urged that the claims are allowable over the prior art and satisfy the provisions of the patent statutes. Reconsideration of this Application and early

notice of allowance are requested. The Examiner is invited to telephone the undersigned to expedite allowance of this application.

Respectfully submitted,

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Amended Copy of Page 1, Paragraph on Lines 2-3; Version with markings to show changes made

METHODS FOR [DIAGNOSTIC] DIAGNOSIS AND/OR TREATMENT OF ANTIPHOSPHOLIPID [ANTIBODIES] ANTIBODY-RELATED DISEASES[,] AND DEVICES THEREFOR

Amended Copy of Page 1, Paragraph on Lines 6-10; Version with markings to show changes made

The present invention relates to [the obtention of] obtaining antibodies recognizing lipids and more particularly, is related to methods for obtaining antibodies against lipidic structures different [to] from the lipidic bilayer, and to the use of these antibodies in diagnostic and/or treatment of diseases associated [to] with the antiphospholipid syndrome; as well as for the determination of physiological states of the cell.

Amended Copy of Page 2, Paragraph on Lines 13-23; Version with markings to show changes made

On the other hand, some studies have demonstrated that the binding of antiphospholipid antibodies to the lipidic antigen increases in the presence of a plasmatic protein. For example, in 1990, McNeil et al., determined that the binding of antibodies to cardiolipin was markedly enhanced by the plasma protein β_2-glycoprotein I or apoprotein H (*Proc. Nat. Acad. Sci. USA* 87:4120-4124). Additionally, some anti-cardiolipin antibodies are bound directly to β_2-glycoprotein I, as was described by Roubey et al. in 1995 (*J. Immunol.* 154:954-960). These findings suggest that the anti-cardiolipin antibodies may recognize either a cryptic epitope on β_2-glycoprotein I exposed on the complex of β_2-glycoprotein I-cardiolipin, or β_2-glycoprotein I alone but with a very low affinity towards the glycoprotein, as was described by Pengo et al. ([199] 1995, *Thromb. Haemost.* 73:29-34).

Amended Copy of Page 6, Paragraph on Lines 15-26; Version with markings to show changes made

To this respect, the presence in sera from patients with the antiphospholipids syndrome of anti-cardiolipin antibodies, a mitochondrial lipid, of anti-nuclear antibodies and of anti-DNA

antibodies, it is indicative of the existence of previous events that cause immunologic damage to cellular membranes, with the disruption of the cells and the exhibition of the intracellular components to the immunologic system, causing the corresponding immunologic reaction that contributes to the development of the syndrome. However, up to now there have not been found studies which allow to determine the events that cause the disruption of the cellular membrane. In other words, with the existent knowledge so far it is impossible to detect the anti-cardiolipin antibodies, the anti-nuclear or even the anti-DNA antibodies before the damage that has been caused to the cell, impeding an early diagnosis and treatment of the illnesses associated with the syndrome.

Amended Copy of Page 7, Paragraph on Lines 17-21; Version with markings to show changes made

[The second hypothesis, consists on assuming that the lipidic particles are formed in the cellular membrane before its destruction, and they would form anti-lipidic particles antibodies that would destroy the membrane, exposing the intracellular components to the immunologic system and giving place later on to the formation of anti-cardiolipin and anti-nuclear antibodies.]

This second hypothesis was proposed in the Master Thesis presented by Monica Lara on August 20, 1999 ("Detection of anti-lipidic particles antibodies in patients with the anti-phospholipid syndrome," Master Thesis, Escuela Nacional de Ciencias Biológicas [National School of Biological Sciences], Instituto Politécnico Nacional [National Polytechnic Institute], Mexico.

Amended Copy of Page 8, Paragraph on Lines 6-12; Version with markings to show changes made

Keeping in mind the deficiencies in the structure and in the molecular association of the antigens that are used in the techniques of induction and detection of antiphospholipid antibodies from the methods of the previous techniques, one of the objectives of the present invention consists on using lipidic antigens with a structure and molecular association similar to the one that is present in patients with illnesses associated with antiphospholipid antibodies, with the purpose of providing a method for the detection of anti-lipidic particles antibodies.

Amended Copy of Page 9, Paragraph on Lines 23-26; Version with markings to show changes made

Figures 5A-5C show the cytofluorometric analysis which indicate that the AC15 serum from a patient with primary antiphospholipid syndrome does not show any immunoreaction with liposomal antigens made from DPPC:PC:[DDPA]DPPA (1.2:0.8:1 mole ratio) that lack lipidic particles.

Amended Copy of Page 9, Paragraph on Lines 30-34; Version with markings to show changes made

Figure 7 illustrates the analysis by the cellular-ELISA method of the reaction between sera from patients with the antiphospholipid syndrome and C5337 human pancreas cancer cells; patients sera were used adsorbed and without any [adsorbtion] adsorption with liposomal antigens made from PC:PA (2:1 mole ratio) bearing lipidic particles induced by calcium.

Amended Copy of Page 11, Paragraph on Lines 17-25; Version with markings to show changes made

Equally, when an antigen that contains lipidic particles is administered in BALB/c mice it has been found surprisingly that these mice developed alopecia and lesions on the face in the form of butterfly wings, similar to those described in some human autoimmune illnesses, as well as deposits of immune [complex] complexes and pathological alterations in their different organs. Additionally, it has been also found that these mice firstly developed anti-lipidic particles antibodies and subsequently anti-cardiolipin antibodies, lupus anticoagulant and anti-nuclear antibodies, which confirms that anti-lipidic particles antibodies constitute the first stage in the development of illnesses associated with antiphospholipid antibodies.

Amended Copy of Page 11, Paragraph on Lines 26-34; Version with markings to show changes made

According to the above-mentioned experiments, the presence of anti-lipidic particles antibodies in one of the first stages of the illness, indicates that one of the first events that [occurrs] occurs in the antiphospholipid syndrome is the formation of anti-lipidic particles antibodies. These antibodies when reacting with lipidic particles in cellular membranes, cause

damage in these membranes, and finally cells disruption and the exhibition of the intracellular components to the immunitary system; which explains the subsequent presence of anti-cardiolipin antibodies, where cardiolipin is a mitochondrial lipid, of anti-nuclear and anti-DNA antibodies, which have been reported as present in these illnesses in humans.

Amended Copy of Page 12, Paragraph on Lines 13-23; Version with markings to show changes made

Therefore, an aspect of the present invention is to develop a diagnosis method for determining if an individual having clinical characteristics of the primary antiphospholipid syndrome (Table 1), or one of the illnesses associated to the secondary antiphospholipid syndrome (Table 1) and who does not present yet anti-cardiolipin antibodies, lupus anti-coagulant, anti-DNA or anti-nuclear antibodies, does have an illness associated to the presence of antiphospholipid antibodies; where such method comprises the steps of detecting in a direct or indirect fashion the presence or absence of lipidic particles in a [serum] sample from said individual, and to observe whether lipidic particles are detected or not, where the presence of said lipidic particles indicates the development of an illness associated to the presence of antiphospholipid antibodies in said individual.

Amended Copy of Page 17, Paragraph on Lines 29-33; Version with markings to show changes made

H) A fifth step of addition and incubation, in which an effective quantity of the peroxidase [sustrates] substrates is added to each one of the wells and said microtiter plate is incubated for 0.1 to 0.5 h at a temperature between 35 and 40°C, stopping the peroxidase reaction by adding an effective quantity of sulfuric acid.

Amended Copy of Page 18, Paragraph on Lines 15-18; Version with markings to show changes made

Liposomal-ELISA method allows the simultaneous determination of anti-lipidic particles antibodies in at least 40 sera samples, each one by duplicate, in a single microtiter plate, therefore, this method can be [easyly] easily applied to the diagnosis of illnesses where this type of antibodies are present.

Amended Copy of Page 20, Paragraph on Lines 26-29; Version with markings to show changes made

D) A second step of [wahsing] washing, in which the antigen bound to the antibody porter is washed with a phosphates buffer solution at pH between 7.0 to 7.4. Preferably repeating 3-times and avoiding that the surface of the cellular culture becomes dry when eliminating the phosphates buffer solution.

Amended Copy of Page 21, Paragraph on Lines 15-18; Version with markings to show changes made

In a specific embodiment, the effective quantity of CO_2 is attained with 1 to 10% in volume with regard to air, while the effective quantity of phosphates buffer solution is attained with 1 to 10 ml. The fluorescent [sustrate] substrate can also be selected from the group consisting of phycoerythrin, Cy3 and Percp.

Amended Copy of Page 22, Paragraph on Lines 22-26; Version with markings to show changes made

H) A fourth step of addition and incubation, in which an effective quantity of peroxidase [sustrates] substrates is added to each one of the wells of microtiter plate, being incubated said plate for 0.1 to 0.5 h at a temperature between 35 to 40°C, stopping the peroxidase reaction by means of an effective quantity of sulfuric acid.

Amended Copy of Page 23, Paragraph on Lines 3-5; Version with markings to show changes made

On the other hand in another specific modality of this method, the second antibody can be conjugated to the enzyme alkaline phosphatase, instead of peroxidase, in this case the corresponding alkaline phosphatase [sustrates] substrates are used.

Amended Copy of Page 23, Paragraph on Lines 6-15; Version with markings to show changes made

Another aspect of the present invention, is to develop an *in vitro* diagnosis instrument for illnesses associated with antiphospholipid antibodies, useful to carry out the method of the

present invention. This diagnosis instrument includes at least an indicator reagent to detect the presence of lipidic particles [and/or] or anti-lipidic particles antibodies in a sample of an individual having clinical characteristics of primary antiphospholipid syndrome (Table 1), or of the illnesses associated to secondary antiphospholipid syndrome (Table 1) and who does not present yet anti-cardiolipin antibodies, lupus anticoagulant, anti-DNA or anti-nuclear antibodies; media to allow the reaction of the sample with the indicator reagent; and, procedures to make evident this reaction.

Amended Copy of Page 23, Paragraph on Lines 16-18; Version with markings to show changes made

In a preferred embodiment, the indicative reagent is selected among liposomes with lipidic particles in their surface, [neoplasic] neoplastic cells, anti-lipidic particles polyclonal antibodies, and/or anti-lipidic particles monoclonal antibodies.

Amended Copy of Page 26, Paragraph on Lines 26-29; Version with markings to show changes made

In a preferred embodiment, the effective quantity of the antigen suspension in the stage A it is of 50 to 100 µl. The second antibody can be also conjugated to the enzyme alkaline phosphatase, instead of peroxidase, in this case the corresponding alkaline phosphatase [sustrates] substrates are used.

Amended Copy of Page 28, Paragraph on Lines 11-14; Version with markings to show changes made

In a preferred embodiment, the antigen suspension is obtained suspending the antigen in a buffer solution at pH between 7.0 to 7.4, in a relationship of 1 to 5 mmole per liter of buffer solution for liposomal antigen. Furthermore, fluorescent [sustrate] substrate can also be selected from the group consisting of phycoerythrin, Cy3 and Percp.

Amended Copy of Page 28, Paragraph on Line 33 through Page 29, Line 25; Version with markings to show changes made

Costar microtiter plates, with 96 flat-bottom wells with a high lipidic antigens binding property (Costar Co. Cambrige, USA), were coated by the addition of 100 µl per well of liposomes made from egg-yolk phosphatidylcholine[.]:phosphatidate (2:1 mole ratio) in Tris-NaCl buffer (10 mM, 1 mM) pH 7, containing 0.1 µmol of phosphatidate, and treated with 5 mM $CaCl_2$ to induce lipidic particle formation. Microtiter plates were incubated 1 h at room temperature. After microtiter plates were incubated they were blocked for 1 h at room temperature by addition of 200 µl per well of 0.4% (w/v) gelatin in Tris-NaCl buffer (10 mM, 1mM) pH 7, containing $CaCl_2$ 5 mM. Then the blocking solution was discarded by suction and 100 µl of human sera, from patients with the antiphospholipid syndrome, at 1:50 dilution using blocking solution were quickly added to each well in duplicate, to avoid that these wells becomes dry; all solutions were added subsequently in the same way. As a positive control, the supernatant of a hybridoma containing a monoclonal antibody against lipidic particles, from IgM isotype, at 1:100 dilution using blocking solution were added to four wells. Human sera were heated previously at 56°C for 30 min for the inactivation of the complement. After microtiter plates were incubated 1h at room temperature they were washed 4-times with 500 µl of blocking solution. Then 100 µl of peroxidase-conjugated goat anti-Fc of human IgG, IgA and IgM antibodies or anti-Fc of mouse IgM antibodies at 1:2000 dilution into blocking solution were added to each well, [respectivelly] respectively, as second antibody. After 1 h of incubation at room temperature microtiter plates were washed 4-times again with the blocking solution and 100 µl of freshly prepared peroxidase [sustrates] substrates were added to each well (10 mg o-phenylendiamine, 25 ml Tris-NaCl buffer (10 mM, 1 mM) pH 7, and 20 µl of 30% H_2O_2) and allowed to incubate in an oven at 37°C for 20 min. Enzyme reaction was stopped by addition of 50 µl per well of 2.5 M sulfuric acid. Absorbances were read at 492 nm in an ELISA Labsystems reader Multiskan MS model; duplicate values were averaged for each serum sample tested.

Amended Copy of Page 30, Paragraph on Lines 22-27; Version with markings to show changes made

Anti-cardiolipin antibodies were detected using cardiolipin coated to ELISA microtiter plates as antigen (Loizou et al., 1985, *Clin. Exp. Immunol.* 62:738-745). Results are also

expressed in Arbitrary Units (AU) and they are considered positive when they have values ≥ 1.9 AU for IgG isotype, and ≥ 2.4 AU for IgM isotype (Loizou et al., *op. cit.*, 1985). All [patients] patients' sera were positive for IgG isotype and some of them were positive for IgM isotype (Table 2).

Amended Copy of Page 32, Complete Page; Version with markings to show changes made

Table 2. Detection of anti-cardiolipin and anti-lipidic particles antibodies in human sera

Healthy blood donators	Anti-cardiolipin Antibodies (ELISA)		Anti-lipidic particles Antibodies (liposomal antigen made from phosphatidylcholine: phosphatidate (2:1) + $CaCl_2$) (Cytofluorometry)	[Patients] Patients' sera [And] and diagnostic		Anti-cardiolipin antibodies (ELISA)		Anti-lipidic particles antibodies (liposomal antigen made from phosphatidylcholine: phosphatidate (2:1) + $CaCl_2$) (Cytofluorometry)		
	IgM (+) ≥ 2.4 AU	IgG (+) ≥ 1.9 AU	Positive results at: (+) D≥0.5, p<0.001 (Polyvalent)			IgM (+) ≥ 2.4 AU	IgG (+) ≥ 1.9 AU	Positive results at: (+) D≥0.5, p<0.001 [Polivalente] Polyvalent	IgM	IgG
1H	-	-	-	AC11	PAPS	-	7.5	D=0.76	-	D=0.54
2H	-	-	-	AC12	PAPS	-	56.3	D=0.77	-	D=0.70
3H	-	-	-	AC13	SLE	5.24	17.2	D=.77	D=0.65	D=0.70
4H	-	-	-	AC14	SLE	-	10.6	D=0.74	D=0.65	D=0.62
5H	-	-	-	AC15	PAPS	-	6.7	D=0.74	D=0.50	D=0.84
6H	-	-	-	AC16	SLE	-	2.52	D=0.75	D=0.56	D=0.59
7H	-	-	-	AC17	SLE	-	4.3	D=0.75	-	D=0.59
8H	-	-	-	AC18	SLE + [P]APS	-	67.4	D=0.73	-	D=0.63
9H	-	-	-	AC19	SLE	-	13.6	D=0.73	D=0.72	D=0.73
10H	-	-	-	AC20	SLE	-	9.3	D=0.75	D=0.52	D=0.73
11H	-	-	-	AC21	PAPS	-	3.36	D=0.75	-	D=0.62
12H	-	-	-	AC22	SLE + [P]APS	2.8	15.4	D=0.56	D=0.52	D=0.61
13H	-	-	-	AC23	SLE + [P]APS	-	19.2	D=0.59	-	D=0.61
14H	-	-	-	AC24	PAPS	-	18.0	D=0.61	-	D=0.61
15H	-	-	-	AC25	PAPS	3.95	16.3	D=0.53	-	D=0.62
16H	-	-	-	AC26	SLE	3.06	9.2	D=0.53	-	D=0.62
17H	-	-	-	AC27	PAPS	-	8.6	D=0.51	-	D=0.54
18H	-	-	-	AC28	PAPS	-	11.5	D=0.51	-	D=0.54
19H	-	-	-	AC29	SLE	-	11.08	D=0.43	D=0.52	D=0.57
20H	-	-	-	AC30	PAPS	-	14.7	N/D	D=0.52	D=0.57
21H	-	-	-	AC31	SLE + [P]APS	-	19.4	D=0.66	D=0.66	D=0.50
22H	-	-	-	AC32	SLE + [P]APS	3.0	39.6	D=0.56	-	D=0.57
23H	-	-	-	AC33	PAPS	-	23.7	D=0.56	-	D=0.54
24H	-	-	-	AC34	SLE + [P]APS	-	34.4	D=0.56	-	D=0.74
25H	-	-	-	AC35	PAPS	4.0	18.0	D=0.66	D=0.56	D=0.75
26H	-	-	-	AC36	SLE	44.0	158.0	D=0.64	D=0.60	D=0.64
27H	-	-	-	AC37	SLE	-	11.0	D=0.64	D=0.70	D=0.75
28H	-	-	-	AC38	PAPS	3.0	2.0	D=0.64	D=0.59	D=0.75
29H	-	-	-	AC39	SLE + [P]APS	-	52.0	D=0.64	D=0.76	D=0.75
30H	-	-	-	AC40	SLE	4.0	18.0	D=0.66	D=0.56	D=0.75

PAPS.- Primary antiphospholipid syndrome. SLE.- Systemic lupus erythematosus. APS +SLE.- Antiphospholipid syndrome secondary to systemic lupus erythematosus

Amended Copy of Page 33, Paragraph on Lines 5-8; Version with markings to show changes made

[Patients] Patients' sera and sera from healthy blood donators were supplied us by Dr. Carlos Lavalle Montalvo, Manager of the Infectology Hospital of the Medical Center "La Raza", from México, D. F., México.

Amended Copy of Page 33, Paragraph on Lines 11-25; Version with markings to show changes made

Reaction of human sera, from healthy blood donators or from patients with the antiphospholipid syndrome, with liposomal antigens made from egg-yolk phosphatidylcholine:phosphatidate (2:1 mole ratio) in Tris-NaCl buffer (10 mM, 1 mM) pH 7, containing 0.1 μmol of phosphatidate, and treated with 5 mM $CaCl_2$ to induce lipidic particles formation, is showed in Figure 1. Immunoreaction of patients sera with the lipidic particles was clearly different from that of healthy blood donators sera, or control sera, since the reaction with the peroxidase [sustrates] substrates was negative when control sera were used. In general, control serum gave values smaller than one AU. All the values from control sera were combined to obtain the arithmetic mean and the standard deviation. We then consider as positive all results greater than 3 standard deviations from the mean. After this analysis, sera from the 30 healthy blood [donators] donors were mixed and the mixture was used as a control sera for subsequent analysis. In Figure 1 the dark line indicates the [uper] upper limit above which the reactions of sera with lipidic antigens are positive. The reaction of most patients sera was clearly positive, with values of AU higher than 6.

Amended Copy of Page 34, Paragraph on Lines 12-23; Version with markings to show changes made

Example 1 was repeated but using as antigens "rigid" liposomes made from dipalmitoylphosphatidylcholine:egg-yolk phosphatidylcholine:dipalmitoylphosphatidate (1.2:0.8:1.0 mole ratio) in Tris-NaCl buffer (10 mM, 1 mM) pH 7, which when incubated with 5 mM $BaCl_2$ they conserve the smooth surface of bilayer. In this case, the reaction of the substrates of peroxidase-conjugated to the second antibody was negative. Because liposomes did not have any lipidic particles, therefore the anti-lipidic particles antibodies did not react with

them and consequently the second antibody did not bind [to] these liposomes, which explains the negative reaction of peroxidase [sustrates] substrates. These results discard the possibility that anti-lipidic particles antibodies recognize a lipid-divalent cation complex and/or the reduction in the liposomal surface charge due to the binding of divalent cations without affecting the bilayer lipid arrangements and their change to lipidic particles arrangements (Aguilar et al., *op. cit.* , 1999).

Amended Copy of Page 34, Paragraph on Line 28 through Page 35 Line 6; Version with markings to show changes made

Example 1 was repeated with some modifications. In this experiment liposomes made from egg yolk phosphatidylcholine:phosphatidate (2:1 mole ratio) in Tris-NaCl buffer (10 mM, 1 mM) pH 7, containing 0.1 μmol of phosphatidate, and treated with 5 mM $CaCl_2$ to induce lipidic particles formation, were incubated directly with peroxidase-conjugated goat anti-Fc of human IgG, IgA and IgM antibodies, or with the supernatant of a hybridoma producing unrelated monoclonal antibodies, as those against a membranal protein of *Trichinella spiralis*, from IgM isotype, and peroxidase-conjugated goat anti-Fc of mouse IgM antibodies. In both cases, the reaction with peroxidase [sustrates] substrates was negative, because in absence of human anti-lipidic particles antibodies or mouse anti-lipidic particles monoclonal antibody the second antibody, peroxidase-conjugated goat anti-Fc of human IgG, IgA and IgM or anti-Fc of mouse IgM antibodies, do not bind directly to lipidic particles induced by calcium in liposomal antigens.

Amended Copy of Page 35, Paragraph on Lines 11-17; Version with markings to show changes made

Example 1 was repeated but in the absence of liposomal antigens, in consequence the reaction of [sustrates] substrates of the peroxidase-conjugated to second antibody was negative. Due to anti-lipidic particles antibodies do not bind directly to microtiter plate which could give a false positive result, because microtiter plate was blocked with the gelatin that is used in this methodology, consequently the second antibody do not bind to microtiter plate which explains the negative reaction of the [sustrates] substrates of peroxidase-conjugated to the second antibody.

Amended Copy of Page 36, Paragraph on Lines 18-31; Version with markings to show changes made

Autofluorescence histograms obtained from egg yolk phosphatidylcholine: phosphatidate (2:1 mole ratio) liposomes showed values between 1 to 10 fluorescence units (a, Fig. 2A). The detection of liposomal autofluorescence allowed the application of cytofluorometry to the analysis of immunologic reactions where liposomal antigens are used. Liposomal autofluorescence (a, Fig. 2A) was not [modify] modified when liposomes were incubated with 5 mM $CaCl_2$ (c, Fig. 2B), which indicates that the presence of lipidic particles in liposomes did not modify the liposomal autofluorescence. Furthermore, this fluorescence was not [also modify] modified by the addition of FITC-conjugated goat anti-Fc of human IgG, IgA and IgM or anti-Fc of mouse IgM antibodies as second antibodies, which indicates that these antibodies do not bind directly [to] liposomal antigens, and therefore they can not produce a false positive reaction. Results with the FITC-conjugated goat anti-Fc of human IgG, IgA and IgM antibodies as second antibody at 1:200 final dilution are shown in: b, Fig. 2A and d, Fig. 2B, with liposomal antigens in absence of calcium (b, Fig. 2A) as in presence of this divalent cation (d, Fig. 2B).

Amended Copy of Page 36, Paragraph on Line 32 through Page 37 Line 4; Version with markings to show changes made

Similar results were obtained with liposomes made from phosphatidylcholine; phosphatidylcholine:cardiolipin (2:1 mole ratio); phosphatidylcholine:phosphatidylserine (4:1 mole ratio) or from dipalmitoylphosphatidylcholine:egg-yolk phosphatidylcholine:dipalmitoylphosphatidate (1.2:0.8:1 mole ratio), respectively. Therefore the cytofluorometry can be applied in general to the analysis of immunologic reactions where liposomal antigens with different lipidic formulations are used.

Amended Copy of Page 37, Paragraph on Lines 26-33; Version with markings to show changes made

Fluorescence readings were made in the FL-1 channel. The relative size and/or liposomal aggregation were analyzed by diffraction of the laser beam in the FSC (forward scatter light) channel and the granularity or liposomal bilayers complexity [were] was analyzed by refraction and reflection of the laser in the SSC (side scatter light) channel. Analysis of 10,000 liposomes

was made in a logarithmic scale with the following detectors: FSC in E00, with a detector compensation threshold of 52 V; SSC of 401 V and FL-1 of 748 V (Baeza et al., *op. cit.*, 1995). The obtained data were analyzed with the Cellquest program (Beckton Dickinson).

Amended Copy of Page 38, Paragraph on Lines 5-21; Version with markings to show changes made

H308 monoclonal antibody do not show any reactivity with bilayer lipid arrangements, since the fluorescence detected from smooth liposomes incubated with this monoclonal antibody (a, Fig. 3A) was similar to the autofluorescence of control liposomes, in Tris-NaCl or treated with calcium, that were not incubated with monoclonal antibody (g, h, Fig. 3D). Furthermore, SSC and FSC values indicated the absence of lipidic particles and of liposomal aggregation in smooth liposomes that were not treated with calcium (i, Fig. 3E; k, Fig. 3F) no matter if they were incubated with H308 monoclonal antibody (c, Fig. 3B; e, Fig. 3C). On the contrary, the 60-fold increase in the fluorescence of liposomes treated with calcium (b, Fig. 3A) with regard to the fluorescence of liposomes with lipids in bilayers (a, Fig. 3A), with a value in the fluorescence difference among these liposomal populations in a logarithmic scale (D)=0.9 at $p<0.001$, showed the reaction of H308 monoclonal antibody with the lipidic particles induced by calcium. Values of $D \geq 0.5$ at $p<0.001$ indicate a difference among the studied populations that is highly significant from the statistical point of view (Lampariello, 2000, *Cytometry* 39:179-188). Therefore, values of $D \geq 0.5$ at $p<0.001$ were considered as positive results and indicative of the presence of anti-lipidic particles antibodies in the analyzed samples.

Amended Copy of Page 38, Paragraph on Lines 30-34; Version with markings to show changes made

Monoclonal antibody reaction with lipidic particles of liposomal antigens is considered as a positive reference of the reaction of [patients] patients' antibodies with this type of lipidic structures. In consequence it is necessary to include this determination as a positive control in the analysis of the detection of anti-lipidic particles antibodies in sera from human individuals or animals by liposomal cytofluorometry.

Amended Copy of Page 40, Paragraph on Lines 5-11; Version with markings to show changes made

Fluorescence readings were made in the FL-1 channel. The relative size and/or liposomal aggregation were analyzed in the FSC channel and the granularity or liposomal bilayers complexity was analyzed in the SSC channel. Analysis of 10,000 liposomes [were] was made in a logarithmic mode with the following detectors: FSC in E00, with a detector compensation threshold of 52 V; SSC of 401 V and FL-1 of 748 V (Baeza et al., *op. cit.*, 1995). The obtained data were analyzed with the Cellquest program (Beckton Dickinson).

Amended Copy of Page 40, Paragraph on Lines 12-16; Version with markings to show changes made

As a negative control, the reaction of healthy blood donators sera with liposomes made from egg yolk phosphatidylcholine:phosphatidate (2:1 mole ratio) in Tris-NaCl buffer (10 mM, 1 mM) pH 7, containing 0.1 μmol of phosphatidate, and treated with 5 mM $CaCl_2$ to induce lipidic particles formation [were] was analyzed. FITC-conjugated goat anti-Fc of human IgG, IgA and IgM antibodies were used as second antibody.

Amended Copy of Page 40, Paragraph on Lines 17-27; Version with markings to show changes made

Thirty healthy blood [donators] donors' sera were studied. These sera did not present any immunoreaction with lipidic particles, since fluorescence graphs obtained with liposomes incubated with them were similar to those of control liposomes that were exclusively incubated with 5 mM $CaCl_2$ (h, Fig. 3D; and j, Fig. 3E from Example 2A). After this analysis, sera from the 30 healthy blood donators were mixed and the mixture was used as a control sera for subsequent analysis. Cytofluorometry graphs of mixed sera are presented in: a, Figs. 4A, D, G, J, M, P, S and V; in c, Figs. 4B, E, H, K, N, Q, T and W, and in e, Figs. 4C, F, I, L, O, R, U and X. FSC values (e, Figs. 4C, F, I, L, O, R, U and X) showed the absence of liposomal aggregation by the treatment with healthy blood donators sera, because they were very similar to those of liposomes control in absence of human sera indicated in: l, Fig. 3F, from Example 2A.

Amended Copy of Page 40, Paragraph on Line 28 through Page 41 Line 11; Version with markings to show changes made

Immunoreaction of all [patients] patients' sera with liposomal antigens treated with calcium showed a fluorescence 20 to 40-fold higher than that of control sera reaction, with a difference between liposomal fluorescence in a logarithmic scale (D) ≥ 0.5 at $p < 0.001$ (Table 2).[.] Values of $D \geq 0.5$ at $p < 0.001$ were considered as positive results and indicative of the presence of anti-lipidic [particles] particle antibodies in sera analyzed, in a similar way as it was described for H308 monoclonal antibodies. As example, fluorescence histograms of eight sera from [patient] patients with systemic lupus erythematosus (SLE) (AC19 and AC20), with primary antiphospholipid syndrome (PAPS) (AC15, AC21 and AC30) or with antiphospholipid syndrome secondary to systemic lupus erythematosus (SLE+APS) (AC18, AC22 and AC31) are [showed] shown in: b, Fig. 4A; g, Fig. 4D; j, Fig. 4G; m, Fig. 4J; o, Fig. 4M; r, Fig. 4P; u, Fig. 4S; and x, Fig. 4V. In the eight sera the reaction between anti-lipidic [particles] particle antibodies contained in [patients] patients' sera and lipidic particles of liposomal antigens although positive, [was] were clearly different [to] from each other and with regard to the reaction of H308 monoclonal antibody (compare d, Fig. 4B; h, Fig. 4E; k, Fig. 4H; n, Fig. 4K; p, Fig. 4N; s, Fig. 4Q; v, Fig. 4T; and y, Fig. 4W with d, Fig. 3B, from Example 2A), which can be [atributted] attributed to the polyclonal [origen] origin of human antibodies.

Amended Copy of Page 42, Paragraph on Lines 4-12; Version with markings to show changes made

"Rigid" liposomes made from dipalmitoylphosphatidylcholine:egg-yolk phosphatidylcholine:dipalmitoylphosphatidate (1.2:0.8:1.0 mole ratio) in Tris-NaCl buffer (10 mM, 1 mM) pH 7, that incubated with $BaCl_2$ 5 mM [they conserve] conserved the smooth surface, were used as antigens. In "rigid" liposomes there were not the formation of lipidic particles because their rigid bilayers do not allow the lipidic movement that is required to form lipidic particles. These liposomes were incubated with sera from patients with the antiphospholipid syndrome and FITC-conjugated goat anti-Fc of human IgG, IgA and IgM antibodies were used at a final dilution of 1:200 as second antibody.

Amended Copy of Page 45, Paragraph on Lines 11-18; Version with markings to show changes made

Fluorescence readings were made in the [FL-1channel] FL-1 channel. Plaquettes relative size and/or plaquettes aggregation were analyzed by diffraction of the laser beam in the FSC channel. Granularity or membranal plaquette complexity was analyzed by refraction and reflection of the laser in the SSC channel. Analysis of 10,000 plaquettes [were] was made with the following detectors: FSC in E00, in a lineal mode with an amplifier gain of 5 V and with a detector compensation threshold of 52 V; SSC of 450 V and FL-1 of 700 V, both in logarithmic mode (Baeza et al., *op. cit.*, 1995). The obtained data were analyzed with the Cellquest program (Beckton Dickinson).

Amended Copy of Page 46, Paragraph on Lines 11-29; Version with markings to show changes made

C5337 pancreas cancer cells were used as antigens, then 1 x 10^5 cells was seeded in each well of a flat-bottom 96-wells microtiter plates, and they were incubated at 37°C in an atmosphere containing 5% CO_2 until cell confluence in the wells reached 100%. After incubation, 200 µl of a blocking solution containing Tris-NaCl buffer (10 mM, 135 mM) pH 7, and 5% fetal calf serum, were added to each one of the wells and microtiter plates were incubated for 30 min at 37°C. Additionally, the blocking solution was eliminated and 100 µl of sera from patients with the antiphospholipids syndrome, or from [healty] healthy blood donators at 1:50 final dilution, using blocking solution, were quickly added to avoid that cells surface becomes dry. All solutions were added subsequently in the same way. After cell cultures were incubated for 30 min at 37°C in an atmosphere containing 5% CO_2, they were washed 3-times with 200 µl of blocking solution for 5 min in each washing. Next, 100 µl of peroxidase-conjugated goat anti-Fc of human IgG, IgA and IgM antibodies at 1:2000 dilution, into blocking solution, were added as second antibody. Microtiter plates were incubated for 30 min at 37°C in an atmosphere containing 5% CO_2. After incubation, microtiter plates were washed as it was indicated and 100 µl of peroxidase [sustrates] substrates were added to each one of the wells and plates were again incubated for 20 min at 37°C. Finally 50 µl of 2.5 M sulfuric acid were added to stop the peroxidase reaction and the absorbency was read at 492 nm in an ELISA Labsystems reader Multiskan MS model.

Amended Copy of Page 47, Paragraph on Lines 27-31; Version with markings to show changes made

This detection was carried out as it was indicated in the Example 3, with the difference that C5337 pancreas cancer cells were incubated with sera from patients with the antiphospholipid syndrome bearing anti-lipidic particles antibodies instead of H308 monoclonal antibody. [Patients] Patients' sera were used at 1:50 dilution and FITC-conjugated goat anti-Fc of human IgG, IgA and IgM antibodies were used as second antibody.

Amended Copy of Page 47, Paragraph on Line 33 through Page 48 Line 2; Version with markings to show changes made

Neoplastic cell cultures were marked with the anti-lipidic particles antibodies from [patients] patients' sera in a similar way as it was described for C5337 pancreas cancer cells in Figure 6, from Example 3, showing the binding of lipidic particles from neoplastic membranes with these anti-lipidic particles antibodies.

Amended Copy of Page 48, Paragraph on Lines 3-6; Version with markings to show changes made

On the other hand, the methodology described in this Example can be applied in an alternative way to the detection of anti-lipidic particles antibodies in [patients] patients' sera when these antibodies have been not yet detected by the procedures indicated in the Examples 1 and 2B.

Amended Copy of Page 48, Paragraph on Lines 13-15; Version with markings to show changes made

In this preferred embodiment of the diagnosis kit, the cell samples coming from the ill individual [is] are made react with anti-lipidic particles monoclonal antibody, in other words, with the indicator reagent.

Amended Copy of Page 48, Paragraph on Lines 23-27; Version with markings to show changes made

In another preferred embodiment of the present invention, a kit for the detection of lipidic particles in cells in different physiologic states coming from a human or animal subject, includes: at least an indicator reagent including [in] at least an anti-lipidic particles monoclonal antibody; at least a buffer solution as a medium to allow the reaction; and fluorescent or enzymatic procedures to make evident this reaction.

Amended Copy of Page 49, Paragraph on Lines 16-23; Version with markings to show changes made

Immunoreaction analysis of mice sera [were] was made by the liposomal cytofluorometry method. Egg-yolk phosphatidylcholine:phosphatidate liposomes (2:1 molar ratio) in Tris-NaCl buffer (10 mM, 1 mM) pH 7, containing 0.1 μmol of phosphatidate, and treated with 5 mM $MnCl_2$ to induce lipidic particles formation were used as antigens. Analysis of 10,000 liposomas was made in logarithmic mode with the following detectors: FSC in E00, with a detector compensation threshold of 52V; SSC of 401 V and FL-1 of 748 V (Baeza et al., *op. cit.*, 1995). The obtained data were analyzed with the Cellquest program (Beckton Dickinson).

Amended Copy of Page 50, Paragraph on Lines 6-16; Version with markings to show changes made

Sera from mice after they were immunized with liposomal antigens treated with manganese showed an immunoreaction that produce a liposomal fluorescence 10 to 100-fold higher than the reaction of mice control sera (Fig. 8C), with values of $D \geq 0.5$ at $p<0.001$. As example, cytofluorometry graphs of the reaction of serum from RB11, RB14 and RB17 mice are showed in Figs. 8 D, 8E and 8F. Reaction between the antibodies of sera from these mice and lipidic particles [altough] although positive, was different for each serum, with values of D=0.9, D=0.91 and D=0.79, respectively, which can be [atributted] attributed to the polyclonal [origen] origin of these antibodies. SSC values from immunoreaction (Figs. 8 D, 8E and 8F) were similar to those of liposomes control incubated with manganese (Fig. 8 A), and they showed the presence of lipidic particles which give the reaction with the anti-lipidic particles antibodies from mice sera.

Amended Copy of Page 51, Paragraph on Lines 13-18; Version with markings to show changes made

Immunoreaction analysis of mice sera [were] was made by the liposomal cytofluorometry method as it was indicated in Example 4. Egg-yolk phosphatidylcholine:phosphatidate liposomes (2:1 molar ratio) in Tris-NaCl buffer (10 mM, 1 mM) pH 7, containing 0.1 μmol of phosphatidate, and treated with 8 mM procainamide to induce lipidic particles formation were used as antigens. Analysis of 10,000 liposomas was made in logarithmic mode as it was described in Example 4.

Amended Copy of Page 52, Paragraph on Lines 3-14; Version with markings to show changes made

Sera obtained after mice were immunized with liposomal antigens treated with the lipidic particles inducer drug procainamide showed an immunoreaction that produce a liposomal fluorescence 10 to 100-fold higher than the reaction of control mice sera (Fig. 10C), with values of $D \geq 0.5$ at $p<0.001$. As example, cytofluorometry graphs of the reaction of serum from RF11, RF14 and RF17 mice are showed in Figs. 10D, 10E and 10F, respectively. Reaction between the antibodies of sera from these mice and lipidic particles[altough] although positive, was different for each serum, with values of D=0.8, D=0.72 and D=0.67, respectively, which can be [atributted] attributed to the polyclonal [origen] origin of these antibodies. SSC values from immunoreaction (Figs. 10D, 10E and 10F) were similar to those of liposomes control incubated with procainamide (Fig. 10A), and they showed the presence of lipidic particles in liposomes which give the reaction with the anti-lipidic particles antibodies from mice sera.

Amended Copy of Page 52, Paragraph on Line 24 through Page 53 Line 2; Version with markings to show changes made

After the detection of anti-lipidic particles antibodies in immunized mice anti-cardiolipin antibodies, anti-nuclear and anticoagulant antibodies were also detected in them, in a similar fashion as it was described for mice immunized with liposomes treated with manganese, from Example 4. These findings corroborate that anti-lipidic particles antibodies constitute the first stage in the development of illnesses associated with antiphospholipid antibodies. Furthermore, in mice immunized with liposomes incubated with procainamide or chlorpromazine it has been

demonstrated the presence of deposits of immune [complex] complexes in different organs. In addition, these mice developed alopecia and lesions in the face in the form of butterfly wings similar to those that have been described in human systemic lupus erythematosus. In Figure 11 the picture of a 7-months age BALB/c female mouse that was treated with liposomes bearing lipidic particles induced by chlorpromazine, where alopecia and lesions in the face in the form of butterfly wings can be observed.

Amended Copy of Page 53, Paragraph on Lines 24-29; Version with markings to show changes made

Immunoreaction analysis of mice sera [were] was made by the liposomal cytofluorometry method as it was indicated in Example 4. Egg-yolk phosphatidylcholine:phosphatidate liposomes (2:1 molar ratio) in Tris-NaCl buffer (10 mM, 1 mM) pH 7, containing 0.1 μmol of phosphatidate, and treated with 8 mM procainamide to induce lipidic particles formation were used as antigens. Analysis of 10,000 liposomas was made in logarithmic mode as it was described in Example 4.

Amended Copy of Page 54, Paragraph on Lines 1-12; Version with markings to show changes made

Sera obtained from mice after they were treated with the lipidic particles inducer drug procainamide showed an immunoreaction that produce a liposomal fluorescence 4-fold higher than the reaction of mice sera before treatment (a, Figs. 12 A, D, and G), with values of $D \geq 0.5$ at $p<0.001$. As example, cytofluorometry graphs of the reaction of serum from RP37, RP38 and RP39 mice are showed in: b, Fig. 12 A; g, Fig. 12 D; and j, Fig. 12 G. Reaction between the antibodies of sera from these mice and lipidic particles [altough] although positive, was different to each serum, with values of D=0.58, D=0.68 and D=0.8, respectively, which can be [atributted] attributed to the polyclonal [origen] origin of these antibodies. SSC values from immunoreaction showed in: d, Fig. 12 B; h, Fig. 12E; and k, Fig. 12H were similar to those of liposomes control incubated with procainamide (Fig. 10A), and they showed the presence of lipidic particles which give the reaction with the anti-lipidic particles antibodies from mice sera.

Amended Copy of Page 54, Paragraph on Lines 18-22; Version with markings to show changes made

Anti-lipidic particles antibodies were also detected before than anti-cardiolipin antibodies, anti-nuclear and anticoagulant antibodies in these mice, in a similar way as it was described for mice in Examples 4 and 4A. Furthermore, the presence of [immune complex] deposits of immune complexes in different organs and the development of alopecia and lesions in the face in the form of butterfly wings were also showed in these mice.

Amended Copy of Page 55, Paragraph on Lines 12-17; Version with markings to show changes made

Immunoreaction analysis of mice sera [were] was made by the liposomal cytofluorometry method as it was indicated in Example 4. Egg-yolk phosphatidylcholine:phosphatidate liposomes (2:1 molar ratio) in Tris-NaCl buffer (10 mM, 1 mM) pH 7, containing 0.1 μmol of phosphatidate, and treated with 5 mM $CaCl_2$ to induce lipidic particles formation were used as antigens. Analysis of 10,000 liposomas was made in logarithmic mode as it was described in Example 4.

Amended Copy of Page 55, Paragraph on Lines 29-33; Version with markings to show changes made

Anti-lipidic particles antibodies were also detected before than [anti-cardiolipine] anti-cardiolipin antibodies, anti-nuclear and anticoagulant antibodies in these mice, in a similar way as it was described for mice in Examples 4, 4A, and 4B. Additionally, the presence of [immune complex] deposits of immune complexes in different organs and the development of alopecia and lesions in the face in the form of butterfly wings were also showed in these mice.

Amended Copy of Page 56, Paragraph on Line 20 through Page 57 Line 2; Version with markings to show changes made

The spleen of the RB14 BALB/c female mouse producing the higher titers of anti-lipidic particles antibodies was removed under sterility conditions and it was placed in a petri dish with 6 ml of incomplete DMEM cell culture medium. Spleen mouse was dispersed until a suspension of single cells was obtaining using blunt tips pincers. Cellular suspension was transferred to a 15-

ml falcon tube and it was left in repose so that the thick residuals settle down. Next, cellular suspension was transferred to another falcon tube and it was centrifuged at 17 x g for 7 min. Subsequently the supernatant was decanted and cellular pellet was resuspended by gently agitation and cellular suspension was diluted by the addition, drop by drop, of 10 ml of incomplete DMEM cell culture medium. Cellular suspension was centrifuged as it was already indicated, then the supernatant was decanted and 4 ml of 0.16 M NH_4Cl were added for erythrocytes lysis. In this step the tube containing cellular suspension was incubated at 37°C and it was gently rotated during 4 min. Later on 6 ml of incomplete DMEM cell culture medium was added and cellular suspension was centrifuged at 17 x g for 7 min. After [centifugation] centrifugation the supernatant was decanted and cellular pellet was gently resuspended in 10 ml of incomplete DMEM cell culture and was allowed to stand at room temperature until their were used (Köhler and Milstein, 1975. Nature 256:495.497).

Amended Copy of Page 57, Paragraph on Lines 3-27; Version with markings to show changes made

On the other hand, P3x63Ag8U.1 myeloma cells were collected from cell culture plates and transferred to falcon tubes. Aliquots from P3x63Ag8U.1 myeloma cells and mouse spleen cells were treated with trypan blue and they were counted in a Neubauer camera. The viability of both cellular suspension were higher than 95%. P3x63Ag8U.1 myeloma cells and mouse spleen cells were mixed in a 1:1 cellular proportion, using 36 x 10^6 cells of each cellular type, later cellular mixture was washed with 10 ml of incomplete DMEM cell culture medium. After centrifugation at 17 x g for 5 min the supernatant was decanted and cellular pellet was gently resuspended. Subsequently, 1 ml of 4000 polyethyleneglycol solution was added drop by drop, during 1 min, and the mixture was manually [shaked] shaken up for 1.5 min, then 1 ml of incomplete DMEM cell culture medium was added for 30 [seg] sec with slow tube rotation. Next, 3 ml of incomplete DMEM cell culture medium was added for 30 [seg] sec also with slow tube rotation, later 16 ml of the same medium was added for 1.5 min with gently agitation. Finally the volume of the fused cell suspension was completed to 40 ml with incomplete DMEM cell culture medium and fused cell suspension was incubated without agitation for 5 min at room temperature. Later on fused cell suspension was centrifuged at 17 x g for 5 min, the supernatant was decanted and fused cell pellet was washed again with 40 ml of incomplete DMEM medium.

Fused cell pellet was resuspended in 30 ml of selection DMEM-HAT medium and aliquots of 100 µl of this fused cell suspension were seeded in each one of the wells of three 96-wells flat-bottom microtiter plates which 24 hs before cell fusion were seeded with macrophages as feeder cells. Microtiter plates were incubated at 37°C, in an atmosphere with 5% of CO_2. After five or eight days of the cellular fusion hybridomas were fed with 50 µl of selection DMEM-HAT medium, finally after 11 days of the cellular fusion hybridoma supernatants were changed by 100 µl of DMEM-HAT media.

Amended Copy of Page 57, Paragraph on Line 28 through Page 58 Line 4; Version with markings to show changes made

After hybridomas growing the supernatants were screened by the liposomal-ELISA method in order to [detec] detect the production of anti-lipidic particles antibodies by them. Cellular samples from all hybridomas producing anti-lipidic particles monoclonal antibodies were [froze] frozen at -70°C in liquid nitrogen. Later, 10 hybridomas with high anti-lipidic particles monoclonal antibodies titers were chosen (Table 3) and they were cloned again by limiting dilution in 96-wells flat-bottom microtiter plates. After hybridomas growing supernatants were screened again by the liposomal-ELISA method and those producing the higher titers of anti-lipidic particles monoclonal antibodies were cultivated in 250 ml bottles for the massive obtention of supernatants containing these antibodies.

Amended Copy of Page 58, Paragraph on Lines 12-22; Version with markings to show changes made

Costar microtiter plates, with 96 flat-bottom wells with a high lipidic antigens binding property (Costar Co. Cambrige, USA) were coated by the addition to each one of the wells of 100 µl of liposomes made from egg-yolk phosphatidylcholine:phosphatidate (2:1 molar ratio) in Tris-NaCl buffer (10 mM, 1 mM) pH 7, containing 0.1 µmol of phosphatidate, and treated with 5 mM $CaCl_2$ to induce lipidic particles formation. Microtiter plates were incubated at room temperature for 1 h and they were blocked for 1 h at room temperature in a similar way as was described in Example 1. Next, blocking solution was discarded by suction and 100 µl of H308 monoclonal antibody that was previously incubated with the [phosphorylate] phosphorylated haptens were added immediately to each one of the wells, to avoid that they becomes dry off.

Amended Copy of Page 59, Paragraph on Lines 3-6; Version with markings to show changes made

Aliquots of 100 µl of H308 monoclonal antibody were incubated with 100 µl of each one of the hapten solutions for 30 min at 30°C. Later on, the liposomal-ELISA method was [applicated] applied as it was described in Example 1. Peroxidase-conjugated goat anti-Fc of mouse IgM antibodies were used as second antibody.

Amended Copy of Page 59, Paragraph on Lines 17-28; Version with markings to show changes made

Inhibition of H308 monoclonal antibody reaction with phosphorylcholine and glycerolphosphorylcholine indicate that the antigen recognition domain in H308 monoclonal antibody has subdomains that recognize specifically the choline methyl groups which lacks ethanolamine and serine (Fig. 13). In addition, total immunoreaction inhibition attained by glycerolphosphorylcholine suggests that the antigen domain that recognize H308 monoclonal antibody include chemical groups of [glicerol] glycerol. These findings are in agreement with the structural pattern proposed for the lipidic particle (Cullis et al., *op. cit.*, 1991) (Fig. 15) where monolayer lipids (C, Fig. 15) that recover the molecular arrangement different to bilayer (B, Fig. 15) are more separate than lipids that constitute a normal monolayer (A, Fig. 15). In an open monolayer (C, Fig. 15) glycerolphosphorylcholine is more exposed than in a normal bilayer therefore this is the region in which the H308 monoclonal antibody reacts.

Amended Copy of Page 59, Paragraph on Line 30 through Page 60 Line 4; Version with markings to show changes made

Possibly the central domain of lipidic particle, the region that is observed as inverted [micela] micella in: B, Fig. 15, is formed by conic shaped lipids such as phosphatidate. In contrast, monolayers most open than a normal monolayer would be formed by phosphatidylcholine and they would be the regions that identify the H308 monoclonal antibody. If H308 monoclonal antibody reacts specifically with a phosphatidylcholine open monolayer, is clear that this antibody does not show any immunoreaction with liposomes formed exclusively by phosphatidylcholine (G, Fig. 14), because in these liposomes the lipids are in a normal

monolayer association that constitute the bilayer, in consequence no immunoreaction with H308 monoclonal antibody is detected.

Amended Copy of Page 60, Paragraph on Lines 15-20; Version with markings to show changes made

Aliquots of 100 µl of [patients] patients' sera that were analyzed in Examples 1 and 2B were incubated with 100 µl of 0.2 µmoles of glycerolphosphorylcholine for 30 min at 30°C. Later on, blocked patients sera were added to the wells of the microtiter plate and the liposomal-ELISA method was [applicated] applied as it was described in Example 1. Peroxidase-conjugated goat anti-Fc of human IgG, IgA and IgM antibodies were used as second antibody.

Amended Copy of Page 60, Paragraph on Line 30 through Page 61 Line 5; Version with markings to show changes made

Studies in BALB/c female mice in which were simultaneously administered the H308 monoclonal antibody, which [develope] developed in these mice a pathology similar to human antiphospholipid syndrome as it was described in Example 4C, and the [gycerol] glycerol-phosphorylcholine hapten, showed a blockage in the development of the pathology in BALB/c female mice. H308 monoclonal antibody was administered by intraperitoneal injection of 1 µg each week during two months to BALB/c female mice, and simultaneously the [gycerolphosphorylcholine] glycerolphosphorylcholine hapten was administered at 2.5 mg/Kg, of body weight, doses by intravenous injection each 24 hs, for 2-months. With this treatment it was inhibited in 40% the development of mice pathology by H308 monoclonal antibody.

Amended Copy of Page 63, Paragraph on Lines 1-3; Version with markings to show changes made

Example 7A. Study by the cytofluorometry method of the cellular membranes stabilization that [prevent] prevents the formation of lipidic particles and the later binding of anti-lipidic particles antibodies.

Amended Copy of Page 63, Paragraph on Lines 12-17; Version with markings to show changes made

Relative size and/or Ag4 mouse myeloma cells aggregation were analyzed in the FSC channel and the granularity or cellular membranes complexity in the SSC channel. Analysis of 10,000 Ag8 mouse myeloma cells [were] was made with the following detectors: FSC in E00 in lineal mode with an amplifier gain of 2 V, with a detector compensation threshold of 52 V, and SSC of 250 V. The obtained data were analyzed with the Cellquest program (Beckton Dickinson).

Amended Copy of Page 63, Paragraph on Lines 18-23; Version with markings to show changes made

Results obtained with Ag4 mouse myeloma cells incubated with the lipidic particles inducer drug chlorpromazine and the lipid bilayer stabilizer drugs spermidine or chloroquine were similar to those described in Figure 16 graphs. These results showed that Ag4 cellular membranes were stabilized by their interaction with lipidic bilayer stabilizers drugs spermidine or chloroquine in consequence they do not [develope] develop lipidic particles with chlorpromazine.

Amended Copy of Page 63, Paragraph on Lines 24-28; Version with markings to show changes made

When Ag4 mouse myeloma cells incubated with both drugs: the lipidic particles inducer drug and the lipidic bilayer stabilizer drug were used as antigens there were not any immunoreaction with the H308 monoclonal antibody, in a similar way as was demonstrated for liposomes [satabilized] stabilized with the drugs spermidine or chloroquine in Example 7.

Amended Copy of Page 63, Paragraph on Lines 29-31; Version with markings to show changes made

These results indicate that cellular membranes of Ag4 mouse myeloma cells stabilized by their interaction with spermidine or chloroquine do not [develope] develop lipidic particles and therefore they do not react with the H308 monoclonal antibody.

Amended Copy of Page 64, Paragraph on Lines 1-7; Version with markings to show changes made

Examples 7 and 7A were repeated using as antigens egg-yolk phosphatidylcholine: phosphatidate (2:1 molar ratio) liposomes, in Tris-NaCl buffer (10 mM, 1 mM) pH 7, and containing 0.1 μmol of phosphatidate, or Ag4 mouse myeloma cells. Liposomal or cellular antigens were incubated with the lipidic particles inducer drug chlorpromazine 0.2 mM for 30 min at room temperature before the treatment with the lipidic bilayers stabilizer drugs spermidine or chloroquine. Stabilizer drugs were used at the concentrations used in Example [7ª] 7A.

Amended Copy of Page 64, Paragraph on Lines 22-34; Version with markings to show changes made

Studies in BALB/c female mice in which were simultaneously administered the H308 monoclonal antibody, which [develope] develop in these mice a pathology similar to human antiphospholipid syndrome as it was described in Example 4C, and one of the lipid bilayer stabilizer drugs spermidine or chloroquine, showed a delay in the development of this pathology. [H3081] H308 monoclonal antibody was administered by intraperitoneal injection of 1 μg each week for 2-months to BALB/c female mice, and simultaneously the stabilizer drug [chlroquine] chloroquine was administered at 2.5 mg/Kg, of body weight, oral doses each 24 hs, during two months. With this treatment it was delayed the development of mice pathology induced by H308 monoclonal antibody. It is possible that modifying the doses of stabilizers drugs that are applied to mice it is possible to prevent the development of these illnesses. The chloroquine dose used was similar to the one used in humans in the treatment of [rheumatoide] rheumatoid arthritis and of systemic lupus erythematosus.